The problem: Attempts at infection control most often fail because pathogens have acquired new genes and alleles that confer antibiotic resistance or allow escape from immune surveillance and vaccine immunity. This exchange is of particular concern in the respiratory tract since DNA is abundant, bacterial populations are diverse, and many important pathogens are naturally competent for DNA uptake and transformation. At present no tools are available to anticipate or prevent this genetic exchange, forcing the responses to be purely reactive - researchers and clinicians can only wait to see which new strains succeed and then attempt to control them after the fact.

Hypothesis: Identifying the functional constraints on natural transformation will allow recombination events in the respiratory tract to be predicted and treatment failures to be prevented.

Transformation is the primary mechanism of genetic exchange between related bacteria. Many respiratory pathogens efficiently take up DNA fragments from respiratory mucosa and recombine these with homologous sequences in their genomes. Respiratory mucosa contains a diverse complex but poorly characterized mixtures of host, commensal and pathogen DNAs, and the properties of these DNAs determine the outcomes of multiple steps of the complex transformation process. In some bacteria specific sequences stimulate the initiation of DNA uptake, and in all the efficiency and extent of homologous recombination depend both on chromosomal features and on the sequence differences between the incoming and resident DNA strands. My laboratory has expertise in the molecular biology, bioinformatics, diversity and evolution of natural transformation in *Haemophilus influenzae*. By combining straightforward DNA uptake and transformation experiments with innovative uses of high-throughput sequencing to analyze complex pools of DNA fragments and recombinant genomes, we will develop and test an algorithm that predicts transformation.

SPECIFIC AIMS

1. Predict DNA uptake. Using our new method for retrieving and sequencing the products of DNA uptake, we will characterize the influence on uptake of every position in the genome. This will be used to predict DNA uptake across diverse *H. influenzae* strains and experimental conditions.

2. Predict transformation. Using Illumina sequencing we can measure the frequency of transformation by every donor-specific sequence variant in the genome. We will use this to disentangle the effects of sequence divergence from other factors affecting recombination and to develop a predictive algorithm for transformation.

3. Test our ability to predict transformation. Culture conditions will be developed that simulate those in the respiratory tract. All of the genetic exchange arising under these conditions will be characterized by Illumina sequencing and compared with that predicted by our refined algorithm.

Outcomes and future directions: The ability to predict genetic exchange will help researchers prepare for new variant strains of *H. influenzae*, and will guide design of interventions that are less vulnerable to these changes. With appropriate data the model can be extended to other competent species, first respiratory pathogens (*Neisseria meningitidis, Streptococcus pneumoniae*), then others (*Vibro cholerae, Helicobacter pylori, Campylobacter jejuni, Staphylococcus aureus*). Finally, understanding the strong uptake biases of *H. influenzae* and *N. meningitidis* may reveal ways to block DNA uptake in the respiratory tract, an intervention likely to directly reduce virulence as well as prevent genetic exchange. We cannot, of course, guarantee successful prediction of genetic exchange events, both because of the intrinsically probabilistic nature of genetic changes and because the relevant microbial communities are not yet thoroughly characterized, but we will define the key parameters controlling these events. Genetic exchange is the biggest challenge to current prevention and therapeutic interventions, and our laboratory is the one best prepared to begin the assault on it.